

## Animal Models

102

**F-SPONDIN (SPONDIN-1) NULL MICE EXHIBIT INCREASED BONE FORMATION, DECREASED OSTEOCLAST FUNCTION AND ACCELERATED OSTEOARTHRITIS**

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**Purpose:** We have previously reported that F-spondin (spondin-1), a neuroregulatory protein, is upregulated by chondrocytes in osteoarthritis. These studies showed that spondin-1, a member of the TSR (thrombospondin) type I class super family, activated latent TGF- $\beta$ 1, which appeared to account for selected *in vitro* effects, including induction of the hypertrophic chondrocyte phenotype. In this study we generated *Spon1* knockout mice to investigate the effect of F-spondin *in vivo* in i) skeletal development and ii) OA progression following surgical destabilization of the medial meniscus (DMM).

**Methods:** Knockout mice were generated in collaboration with the Texas Institute of Genomic Medicine by targeted deletion of exon 1 in C57BL/6 mice. Exon deletion was confirmed by Southern blot analysis and PCR using probes specific for wild type (WT) and mutant loci. Total TGF- $\beta$ 1 was detected using R & D ELISA kit. MicroCt was performed on the Scanco mCT 35 system on proximal tibia and femurs bone evaluation. Values represent the average of 10 WT and 11 KO mice. To assess the role of *Spon1* on OA progression following destabilization of the medial meniscus was performed on 3 month old WT and *Spon1*<sup>-/-</sup> mice.

**Results:** *Spon1*<sup>-/-</sup> null mice were viable and initial macroscopic observations revealed no overt differences in size and body weight compared with WT (mice up to 6 months). Since we previously reported spondin-1 to regulate TGF- $\beta$  activity, we measured TGF- $\beta$ 1 serum levels in adult mutant mice. Relative to WT, *Spon1* deletion reduced serum levels of total TGF- $\beta$ 1 ( $82 \pm 20$  ng/ml vs.  $30 \pm 25.0$  ng/ml;  $p < 0.002$ ). Similarly, cultured chondrocytes isolated from the rib cages of 5 day old *Spon1*<sup>-/-</sup> mice also produced significantly less TGF- $\beta$ 1 (30%) compared to WT controls ( $p < 0.01$ ). To determine whether *Spon1* deletion affected bone phenotype, we performed microCT of tibia and femurs in mutant and WT mice aged 1-6 months. Relative to WT mice, *Spon1*<sup>-/-</sup> exhibited increased bone formation at 6 months (Figure 1), evidenced by, a) increased trabecular and cortical bone volume fraction (Bone volume/Total volume:  $0.26 \pm 0.03$  versus  $0.16 \pm 0.05$ ,  $p < 0.0002$ ; Fig 1), b) decreased trabecular spacing ( $0.14 \pm 0.02$  versus  $0.19 \pm 0.03$ ,  $p < 0.0003$ ); and c) increased trabecular number ( $7.7 \pm 1.2$ , versus  $5.5 \pm 0.9$ ,  $p < 0.0005$ ). Interestingly, no significant changes were observed at 1 or 3 months, suggesting that *Spon1* effects are age-dependent. Histologically, tibia from *Spon1*<sup>-/-</sup> mice displayed increased bone ingrowth in the bone marrow cavity, particularly within the spongy bone of the epiphyseal regions. This was accompanied by decreased TRAP staining compared to WT mice, suggesting decreased numbers of osteoclasts. Supporting this observation, osteoclast differentiation, performed by RANKL, M-CSF induction of non-adherent bone-marrow cells, revealed impaired differentiation in *Spon1*<sup>-/-</sup> mice compared to WT.

Preliminary analyses suggest that *Spon1* deletion also accelerated cartilage degradation in the DMM model of osteoarthritis. The increased severity of osteoarthritis-like cartilage destruction 7 weeks post surgery was by accompanied by increased thickening of subchondral bone (Figure 2).

**Conclusions:** Our studies indicate that spondin-1 (F-spondin), a latent TGF- $\beta$ 1 activating ECM protein, over-expressed in OA bone and cartilage, regulates bone metabolism in aging mice. *Spon1*<sup>-/-</sup> mice exhibit increased trabecular and cortical bone formation, decreased osteoclast function and increased susceptibility to surgical induced OA. Together these data suggest that a primary function of spondin-1 in skeletal tissue is the regulation of bone mass via latent TGF- $\beta$ 1 activation. Further studies are in progress regarding the potential of spondin-1 as a drugable target in future therapy of osteoporosis or osteoarthritis.

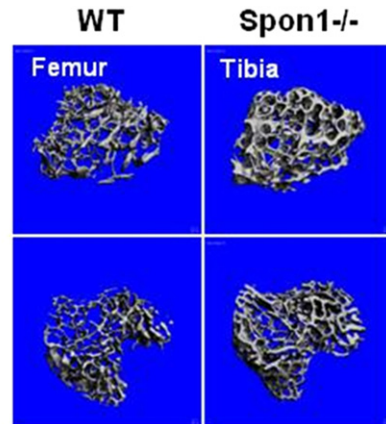


Figure 1

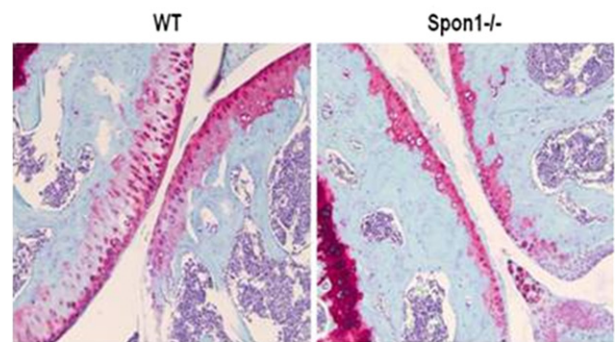


Figure 2

103

**GENE ARRAY PROFILING OF ARTICULAR CHONDROCYTES IN MICE WITH DIFFERENT SUSCEPTIBILITY TO NATURAL DISEASE REVEALS SPECIFIC GENE SIGNATURES LINKED TO HEALTHY AGEING AND SPONTANEOUS OA.**

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**Purpose:** The mechanisms initiating and driving spontaneous osteoarthritis (OA) progression remain largely unknown. The Str/ort mouse strain has high natural susceptibility to OA with ageing, whereas CBA mice show little, if any, spontaneous OA development. In this study, we used this inter-strain variability to spontaneous OA in mice at various ages to determine articular cartilage (AC) gene expression patterns and biological pathways that contribute to normal, healthy and osteoarthritic AC ageing and that play a role in susceptibility to OA.

**Methods:** AC from Str/ort and CBA mice at 8 (before overt OA in Str/ort), 18 (early OA) and 40wks of age (late OA) were isolated from tibial and femoral knee joint condyles and RNA extracted (n=4 per group) for gene expression microarray analysis. Ingenuity® pathway analysis was performed for each comparison: 8 vs. 40wks CBA, 8 vs. 40wks Str/ort, 8wks CBA vs. Str/ort, 8 vs. 18wks Str/ort. RT-qPCR was performed on selected genes.

**Results:** Profiling in 8 and 40wk-old CBA mice was used to pinpoint gene signatures linked to healthy AC ageing. This comparison revealed 190 differentially-regulated genes (50 up, 140 down), which did not include any genes previously linked to OA, except CILP and HDAC5. Pathway analysis revealed that skeletal muscle-specific genes were significantly down-regulated in the AC of CBA mice with ageing from 8-40wks. Changes in this

muscle-related gene signature were not, however, seen in ageing Str/ort mice and these differences confirmed by RT-qPCR for *Myf6* and *Myh1*. Comparison of Str/ort mice at these same ages, during which OA develops, showed 279 differentially regulated genes (47 up, 232 down) and these were mainly centred on the signalling via the NFkB complex.

To determine the gene expression patterns related to OA susceptibility, 8wk-old CBA and Str/ort mice AC were compared. This showed up-regulation of 139 genes (none down-regulated), including *Htra1* and *TIMP1*, which was again centred primarily on NFkB pathway signalling. Immunolabelling of AC sections showed that the NFkB-p65 protein was more highly expressed in chondrocytes of 8wk-old Str/ort than in aged-matched CBA mice.

To define gene profiles specific to early OA, AC from 8 and 18wks old Str/ort mice was compared. This showed 113 down-regulated and only 2 up-regulated genes and pathway analysis showed that these were again centred on the NFkB pathway. Interestingly, no genes were differentially regulated between 18 and 40wks in Str/ort mice, suggesting that similar processes are taking place in both early and late-stage OA.

**Conclusions:** We find that AC chondrocytes express skeletal muscle-related genes in young mice that are lost during normal healthy ageing but retained during OA. In addition, our data support the involvement of NFkB pathway signalling in susceptibility of AC to spontaneous OA and to OA progression. This study highlights these molecular processes as possible markers of OA and targets for slowing OA development.

#### 104

##### GENERATION OF AN INDUCIBLE CARTILAGE SPECIFIC DELETER USING HUMAN AGGREGAN ENHANCER/PROMOTER THAT IS TRACKABLE IN VIVO USING LUCIFERASE

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**Purpose:** To generate a chondrocyte-specific deleter which can be visualised in vivo. We needed an improvement on the collagen type II which is significantly reduced in adult articular cartilage and expresses in kidney during development

**Methods:** We have utilized a transgenic approach where we have used the human aggrecan enhancer/promoter to drive inducible Cre recombinase (Cre-ERT2) followed by an IRES luciferase forming a bicistronic mRNA in transgenic mice.

**Results:** The expression and efficiency of the inducible cre recombinase was tested by examining X-gal staining of tissues from embryos as well as adult in double transgenic with Rosa 26R mice. Cre recombinase was induced by tamoxifen, at different time points during development and postnatally. X-gal staining was observed in growth plate and articular cartilage as well as the fibrocartilage of meniscus, trachea, and intervertebral discs reproducing the pattern of endogenous aggrecan gene expression.

In addition to this mouse being an efficient deleter, the presence of luciferase allows the visualization of aggrecan expression in vivo. This has been tested before or after the induction of osteoarthritis through destabilisation of the medial meniscus ligament up to eight weeks post surgery.

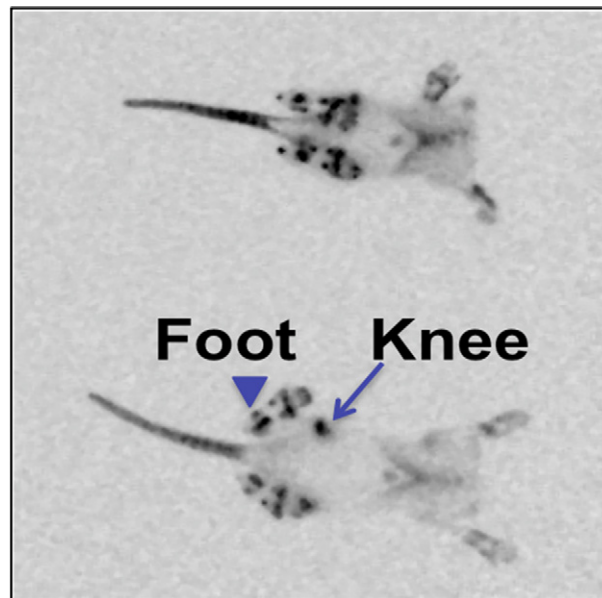
**Conclusions:** The aggrecan-CreERT2 will help us determine genes involvement in the integrity of the cartilage and visualize this complex in osteoarthritis

#### 105

##### DEFICIENCY OF NFAT1 TRANSCRIPTION FACTOR CAUSES OSTEOARTHRITIS WITH ALTERATIONS IN ARTICULAR CARTILAGE AND SUBCHONDRAL BONE IN ADULT MICE

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**Purpose:** One of the barriers to progress in OA research is the difficulty in obtaining human joint tissue samples at an early stage of OA that permits studies of the mechanisms for initiation of OA. Many animal models have been developed to explore the mechanisms of human OA. However, surgically- or chemically-induced OA models often display rapidly progressive joint lesions and may not be suitable for studying the etio-pathogenesis of more slowly progressive non-traumatic OA in humans. Murine models of OA developed by spontaneous or genetically-induced



mutations of genes for cartilage matrix proteins or growth factors are often accompanied by developmental defects in the skeletal system (e.g., mice harboring mutations in aggrecan or type-II collagen). Notably, these mouse models usually do not show subchondral bone changes, an important pathogenetic feature of human OA. Therefore, there is a crucial need for animal models that mimic the pathologic characteristics of idiopathic human OA to study the mechanisms for initiation and progression of OA.

**Methods:** We examined whether deletion of *Nfat1*, a transcription factor previously reported as a regulator of the expression of cytokine genes during the immune response, would alter the expression of specific proinflammatory cytokines and display osteoarthritic changes in articular tissues of mice.

**Results:** The results revealed that deletion of *Nfat1* transcription factor in mice caused classic OA changes, including alterations in articular cartilage and subchondral bone that mimic those in human OA. *Nfat1*-deficient mice exhibited normal skeletal development but displayed loss of type-II collagen and aggrecan in young adult articular cartilage of load-bearing joints. These early changes were followed by articular chondrocyte proliferation/clustering, thickening of subchondral bone, slow progression of articular surface destruction, and formation of chondro-osteophytes. Overexpression of specific matrix-degrading proteinases and proinflammatory cytokines was observed in *Nfat1*-deficient articular cartilage and synovium. We identified *Nfat1* binding sites in the promoters of the genes for mouse and human interleukin-1 $\beta$ , tumor necrosis factor- $\alpha$ , matrix metalloproteinase-13 (MMP13), and a disintegrin and metalloproteinase with thrombospondin motifs-4,5 (ADAMTS-4,5), suggesting that *Nfat1* may be an upstream regulator of these catabolic molecules that degrade articular cartilage.

**Conclusions:** These novel findings suggest that *Nfat1*-deficient mouse may serve as a more suitable surrogate of human OA than murine OA models that do not exhibit subchondral bone changes. Because *Nfat1* regulates multiple matrix-degrading proteinases and proinflammatory cytokines in articular tissues, anti-OA agents that target *Nfat1* could be more effective than drug candidates that target a single catabolic molecule.

#### 106

##### EARLY INTERVENTION TO PREVENT CARTILAGE DEGENERATION BY ADMINISTRATION OF ANTI-VEGF ANTIBODY IN RABBIT MODEL

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**Purpose:** We have previously constructed and transplanted scaffold-free tissue-engineered cartilage into an osteochondral defect (Tissue Eng Part A 2008;14:1183-1193), and confirmed that reparative cells derived from